Example 5

Increased Transfection Efficiency and Expression of E25 Antibody in Transient Transfections Using Caspase-9-DN

Serum free adapted CHO DP12 cells were seeded at 1.5 million cells/ml in untreated 12 well tissue culture plates in medium based on DMEM/HAM F-12 with modified concentrations of some components and containing recombinant human insulin, trace elements and serum. Transfection was performed using DMRIE-C (Gibco BRL) according to manufacturer's instructions. Caspase-9-DN expressing clone 14 was transfected next to controls which were CHO DP12 cells and E25 cells (CHO DP12, expressing E25).

Red shifted GFP expressing vector (Quantum Biotechnologies Inc.) was co-transfected with a DNase expressing vector [Shak, S. et al., (1990), *Proc Natl. Acad. Sci USA,* 87:9188–9192)]. 24 hours post-transfection, propidium iodide was added to an aliquot of the culture and total and viable transfection efficiencies were assayed by flow cytometry on FACSCalibur (Becton Dickinson). Five days after transfection, a sample of the medium was subjected for DNase titer analysis using ELISA.

The data indicated (FIG. **13**) that transfection reagent, in our experiment DMRIE-C, can be toxic to cells when used at higher concentrations (above 6 ul). In FIG. **14**, caspase-9-DN clone 14 shows (in all concentrations of DMRIE-C tested) higher total and viable transfection efficiencies than controls. The transfection efficiency of clone 14 increased with the amount of transfection reagent and reached maximum at 12 ul of DMRIE-C, at which concentration both controls already started to show a decrease in transfection efficiency. It is possible that transfection efficiency of clone 14 will increase even further when higher than currently tested amount of DMRIE-C is used. The increase in transfection efficiency of clone 14 was reflected in the specific productivity (DNase titer/total LDH) of the culture and in DNase titer (FIGS. **15**, **16**), both of which were increased up to four-fold compared with the controls.

Example 6

Effect of Caspase 9-DN Expression on Viability and Viable Cell Number After Thawing a Frozen Culture

2×10^7 cells of caspase-9-DN expressing clone 14 and E25 control cells were frozen in a freezing medium (1 g/L methylcellulose in modified DMEM/Ham F-12 and 10% DMSO) and stored at −80° C. for an extended period of time. On the day of the experiment, vials of frozen cells were taken out of the freezer, thawed at 37° C. and added to a spinner with a pre-warmed growth medium (modified DMEM/Ham F-12). Cells were cultured for 8 days and assayed for viability and viable cell density.

The results are shown in FIGS. **19** and **20**. The results indicate that caspase-9-DN expressing cells maintained higher viability and viable cell count than the control E25 cells. Thus, expression of caspase-9-DN in the CHO cells had a beneficial effect on viability and viable cell densities upon thawing the frozen cell cultures.

Example 7

Caspase-9-DN Expressing Cells Show Resistance to Butyrate

The following study was conducted to examine whether caspase-9-DN expression affects resistance of the cells to potential adverse effects of butyrate.

Caspase-9-DN expressing clone 14 and E25 control cells were seeded at 1×10^6 cells/ml in 60 mm tissue culture dishes. Each dish contained 4 ml of culture medium. Cultures were grown at 37° C. in concentrated medium based on DMEM/Ham F-12 with insulin and trace elements. Cultures were temperature shifted to 33° C. on the second day and butyrate was added on the third day at varying final concentrations (0, 1, 2, 3, 5, 10 mM) (n=2). Viability of the cultures and titers were assayed daily.

The results are shown in FIGS. **21** and **22**. The results showed that E25 control cells lose viability more rapidly than caspase-9-DN expressing cells (see FIG. **21**, day 7 and day 9). This is reflected in the titers of protein of interest. Titers shown in FIG. **22** indicate that caspase-9-DN cells gave higher titers than 0 butyrate addition in cultures with 1 ,2 ,3 and 5 mM butyrate. On the other hand, titers of E25 controls improved with only 1 and 2 mM butyrate. The results suggest that caspase-9-DN expression protects cells from adverse effects of butyrate and can result in extended viability and higher titers.

What is claimed is:

1. A method of making recombinant proteins using one or more apoptosis inhibitors, comprising the steps of:

(a) providing a vector comprising a gene encoding caspase-9 dominant negative protein,

(b) providing a vector comprising a gene encoding a protein of interest,

(c) providing a Chinese hamster ovary (CHO) host cell,

(d) transforming or transfecting the host cell with the vector of steps (a) and (b),

(e) providing cell culture media,

(f) culturing the transformed or transfected host cell in the cell culture media under conditions sufficient for expression of the protein of interest and the caspase-9 dominant negative protein, and optionally

(g) recovering or purifying the protein of interest from the host cell and/or the cell culture media.

2. The method of claim **1** further comprising the step of admixing an additional apoptosis inhibitor into the cell culture media in steps (e) or (f).

3. The method of claim **1** wherein the vector of step (a) and the vector of step (b) are the same vector.

4. The method of claim **1** wherein the vectors of steps (a) and (b) are two separate vectors.

5. The method of claim **4** wherein the vectors of steps (a) and (b) comprise different antibiotic resistance selection markers.

6. The method of claim **1** wherein the host cells are cultured under conditions for transient expression of the protein of interest.

7. The method of claim **1** wherein the protein of interest comprises a protein which is capable of inducing apoptosis in a mammalian or non-mammalian cell.

8. The method of claim **1** wherein said cell culture media is serum-free media.

9. The method of claim **1** wherein said cell culture media comprises butyrate.

10. The method of claim **1** wherein after step (f), the host cell(s) and/or cell culture media is frozen and subsequently thawed.

11. A method of making recombinant proteins using one or more apoptosis inhibitors, comprising the steps of:

(a) providing a vector comprising a gene encoding a protein of interest,

(b) providing a Chinese hamster ovary (CHO) host cell comprising a gene encoding caspase-9 dominant negative protein,